Development and Evaluation of Ophthalmic Drug Delivery System for Glaucoma

Synopsis

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1. Introduction

Glaucoma is 2nd principal cause of global vision impairment, which affects approximately 85 million people (Barse et al., 2016). As per WHO, approximately 4.5 million population are blind due to glaucoma. Worldwide, primary angle glaucoma is in more prevalence than secondary and also more than 60% populations are more influenced to open angle glaucoma (Addepalli et al., 2019). It is one of the serious ophthalmic complications characterized by rise in intraocular tension, which gradually leads to damage optic nerve and irreversible vision loss. Advancement in glaucoma may be seen because of the disparity among drainage and secretion process of aqueous humour within ocular chamber (Barse et al., 2018). Predisposing factors of the development of glaucoma may be genetic make-up, aging, exogenous and endogenous factors. The main purpose of anti-glaucoma therapy is to decrease enhanced intraocular tension.

In market majority (>90 percent) of ophthalmic dosages are presented in eye drop form. However, they are washed out from the eye surface due to lower contact time to the cornea and results in poor ocular availability (< 5%) after topical application (Morsi et al., 2016). These conventional eye drops are not capable to provide sustained action for prolong time. Different types of ophthalmic preparations including eye drop solutions, gels and ointments have been utilized with an intention to enhance the ocular residency of drugs. Precorneal retention time has been shown to increase with certain formulations. However, because of the impaired vision (ointment) and lower patient compliance, these formulations (ocular inserts) have not been widely embraced (Silva et al., 2012).

Novel drug carriers including nanoparticles (polymeric and lipidic nanoparticles), ocular inserts, liposomes, and mucoadhesive polymers have been utilized to enhance bioactives permeation through the corneal surface. Generally, insertion of large ocular inserts is difficult in placement as non-dissolving delivery system, which is more problematic (Barse et al., 2016). A usual nanodispersion exhibits a low viscosity and as a result leads to low availability of bioactive in the aqueous humor owing to fast precorneal elimination of bioactive compounds from site of action. Formulation like ointment(s) with high viscosity is difficult to instill in the eyes.

To overcome the problems related to ocular inserts, nanodispersion, ointments and eye drops, with poor availability of drug in aqueous humor or other ocular tissues is an implementation of hybrid system of nanoparticles and *in situ* gel (Silva, et al., 2012). From a patient compliance perspective, liquid drug products that are in proximity with ocular area for a prolonged period with sustained-release of drug proved to be best formulations. Therefore, PLGA is of great choice as a polymer matrix due to its biodegradable, bio adhesive and biocompatible property (Almeida et al., 2019). Typically, nanoparticles dispersion is less viscous and they possess less stability therefore, it removes rapidly from precorneal pocket (Morsi et al., 2016). These characteristics can be accomplished by the incorporation of a phase transition system-based delivery approach (Gupta et al., 2010). *In situ* systems allow sustained action, enhanced retention in eye and less drainage from eyes (Ahmed and Goli, 2018; Patil et al., 2012). *In situ* gelling based drug carrier's system is modern drug carrier technology advancement, which encompasses profits of both gels, and solutions (sols) for the purpose of an accurate dose administration, with easy instillation (Srividya et al., 2001).

In ocular drug delivery, for phase transition from sol to gel, a number of triggering forces are used, which include thermo responsive, pH activated, and ion sensitive system (Sheshala et al., 2015). *In situ* gelling triggered by temperature can be accomplished by employing a polymeric material that exists as a solution at normal room temperature that may transform to its gel form at ocular surface (Shastri et al., 2006). Poloxamer, available in the market as Pluronics®, is frequently used thermo-responsive polymeric material in drug products. These synthetic polymers are composed of polyoxyethylene oxide and polyoxypropylene oxide in the center surrounded by a hydrophobic polyoxypropylene oxide backbone (Quintanar-Guerrero et al., 1996). TPGS is a hydrophilic substance is a derived product of Vitamin E (Vit-E) (Mu and Feng, 2002; Alkholief et al., 2019). In the present work TPGS was used as an emulsifier as well as a matrix component to blend PLGA for manufacturing of nanocarrier for control release.

Brimonidine tartrate (BRT) is a frequently prescribed therapeutic candidate in the management of glaucoma, which is a selective α-2 adrenomimetic agent. On reducing IOP, BRT is 100 folds more efficient than apraclonidine. Moreover, neuroprotective

action of BRT has been identified in rabbits and human eyes (Burke and Schwartz, 1996). It shows a dual mechanism in reducing IOP i.e. decreasing the production of aqueous humour with enhanced uveo-scleral outpour (Cantor, 2006; David, 2001). A few scientific studies have been done on BRT prolonged release formulations for the effective therapy of glaucoma. Lately, sustained release of BRT from layered double hydroxide nanoparticles/ thermogel into copolymer-based contact lens (Aburahma and Mahmoud, 2011), liposomes (Prabhu et al., 2010), and eudragit nanoparticles (Bhagav et al., 2011) have been investigated.

Nanoprecipitation method offers the advantage of manufacturing a huge amount of product in a short time, and is practically easy to assemble. It represents a reproducible, robust and uncomplicated technique. It has been a frequently used approach of manufacturing PLGA based nanoparticles. Nanoprecipitation technique is simple, less energy intensive and widely used; sometimes it is used without any additives like surfactants, thickening agents, etc (Hornig et al., 2009). Fundamentally, it involves bottom-up approach and interfacial deposition process for the formation of nanoparticles. BRT is a highly water-soluble drug and does not permeate rapidly through the tight junctions of the cornea. The different strengths (0.1, 0.15, and 0.2 % w/v) of BRT eye drop formulations available in the market but all they have lower precorneal retention time with poor patient compliance (Aburahma and Mahamoud, 2011).

Over conventional eye drops, eye ointments offer some advantages. But because of less patient compliance like obscured vision these have not been generally accepted (Chiou and Watanabe, 1986; Swarbrick and Zhong, 2013). These issues might be overwhelmed by the utilization of phase transition-based gel shaping frameworks, which are imparted by drops to cavity of the eye, and experiences a conversion sol to gel change in the eye environment (Gupta et al., 2007). Embodiment of hydrophilic medications in the nanosized form improves the visual medication maintenance and transcorneal transport, which further will result in improvement of intraocular bioavailability.

In present study BRT-PLGA/BRT-PCL nanoparticles were prepared for the improvement of drug ocular bioavailability. To prevent precorneal rapid drainage,

nano-dispersion was loaded into a thermoreversible based formulation of *in situ* gel (phase transition system). As far as we know, such formulations have not been prepared previously. These pharmaceutical dual approaches are expected to improve the stability of formulation and also provide sustained action for long time in glaucoma therapy.

2. Literature review

Over the last several decades, controlled drug delivery technology has advanced significantly. With the launch of the 1st prolonged-release preparation in 1952, this development began.

2.1 Glaucoma

Pro-niosomal gel formulations were developed that demonstrated sustained release by altering water soluble property of antiglaucoma drug like dorzolamide. In rabbits, an intraocular pressure (IOP) (pharmacodynamic) study was carried out for comparing the modified novel gel formulation with conventional eye drop (TrusoptVR®). The findings revealed that the independent variables investigated significantly impacted entrapment efficiency percent and particle size. An *in vivo* pharmacodynamic research discovered gel was capable of causing a significantly higher reduction in IOP. Furthermore, it was discovered that dorzolamide bioavailability was significantly increased as well as superior to TrusoptVR® eye drops (Fouda et al., 2018).

Vasoregulating activity of antioxidant-tocopherol on the retina by virtue of mediator protein kinase C pathway was investigated. In a study, 30 glaucomatous patients (60 eyes) were included. At the beginning, sixth, and twelfth months of the study, each subject's disease progression was assessed by measuring visual field and colour doppler imaging of posterior ciliary arteries were observed (Engin, 2009).

Lee and associates looked into other studies of simultaneous use of brimonidine and timolol has demonstrated which isn’t inferior to other methods. Concurrent brimonidine and timolol are given twice daily; on the other hand, knowingly less effective in lowering IOP than a fixed combination of timolol 0.50% and latanoprost 0.005% was investigated (Lee and McCluskey, 2008).

2.2 Brimonidine Tartrate And Vitamin E Based Ocular Drug Delivery System

BRT loaded nanoparticles and thermogel carrier containing brimonidine had a 144-hour release effect, which was significantly longer than nanoparticles. Novel thermogel DDS was found biocompatible with corneal epithelium and does not produce any cytotoxic effects. Contact lens using brimonidine thermogel was found to release the drug for 7 days (Sun et al., 2017).

Barwal and associate in their study encapsulated BRT in small-sized chitosan loaded nanoparticles (nano brimonidine). The nanoparticles were evaluated for entrapment efficiency (EE), storage stability, monodispersity and freeze-thawing capability. The evaluated formulation has shown good drug loading capacity, reduced side effects and improved efficacy. Under the electron microscope, glaucoma patients trabeculectomy tissue treated with nano brimonidine showed improved dilation of the trabecular meshwork. This is direct evidence for improved nano brimonidine availability following topical administration. As a result, the newly developed nano brimonidine has the ability to enhance efficacy, reduced dosage and frequency, and focus on improving drug permeation to the frontal region of the eye (Barwal et al., 2019).

Application of brimonidine tartrate (BRT) in gel form was the only proven treatment in persistent facial erythema, which is a pathological feature of rosacea. The topical bioavailability of novel BRT gel and conventional BRT drop was compared. A pharmacokinetic study was carried for comparison of intra individual systemic exposures after use of BRT gel on a dermal layer (0.07 percent, 0.18 percent, and 0.5 percent). The use of 0.2 percent BRT has been advised to patients having medium to serious level of facial erythema associated with rosacea. The gel having concentration 0.5% produces less systemic effect compared with BRT solution. Hence, novel gel exhibits better systemic safety in comparison with other ophthalmic solution (Benkali et al., 2014).

Rimple and Newton developed BRT nanoemulsion by combining surfactants and co-surfactants that increases the permeability through barriers, as result of which it showed faster onset of action and therapeutic effect (Rimple and Newton, 2018).

Barse and co-workers developed, optimized, and conducted an *in vivo* and *ex vivo* study of novel BRT *in situ* phase transition system in comparison to conventional eye

drops for glaucoma treatment. BRT concentration obtained from donor chamber of corneal was 12.40 percent (124 µg). In excised corneal permeation study, BRT concentration obtained from receptor chamber was 76.8 percent (760 µg), after 5h of administration of 1 mg dose. The overall recovery rate from permeation experiment was 89.2 percent. In an *in vivo* study, the gel produces by blending various proportion carbopol 974 and HPMC K4M, significant prolonged IOP reduction as compared with the marketed ophthalmic solution (Barse et al., 2018).

Saylor and co-workers investigated the evidence for BRT's neuroprotective properties in the optic nerve and retinal injury (Saylor et al. 2009). In addition, a search of the PubMed database repository yielded the references for this study. There were 48 articles that addressed one of four neuroprotection criteria. From the literature data, it was confirmed the role of BRT in neuroprotection and fulfils the first three criteria: receptor on the specific cell, transportation into vitreous part and back of the eye including the retina, and creation of intracellular changes in animal models that results increase in neuronal resistance to insults/interrupt cell damage. BRT could not be able to succeed to achieve the final neuroprotective success criterion in human being.

Fudemberg and colleagues studied the efficacy, safety, and current applications of brimonidine. They looked at the research on brimonidine's efficacy and adverse effects, as well as its involvement in glaucoma treatment. Brimonidine is an essential component of topical glaucoma treatment (Fudemberg et al., 2008).

A preclinical study on brimonidine was conducted, which demonstrated that it is one of the potent adrenoceptor agonist. Brimonidine was several folds selective than other adrenoreceptor agonist (clonidine, aminoclonidine, apraclonidine etc). Brimonidine has ability to reduced IOP in different kind of animals, which is unlike for apraclonidine. Brimonidine was found to be neuroprotective in the mechanical injured eye of rat. It can also protect the eye optic nerve and hence prevent secondary damage of eye. Brimonidine was also found safe and nontoxic in ocular use (Burke and Schwartz, 1996)

To increased systemic stability of berberine chloride nanoparticles were modified with vitamin-E TPGS (1%w/v). Nanoparticles with and without surface modified form were brought in exposed to buffer of different pH conditions (7.4 and 6.8) bile

salt and plasma. Because of the steric stabilization feature, vit-E TPGS coated nanoparticles were more stable in different buffers than plain NPs. Surface coated NPs produces comparatively very less effect on hemolysis (Vuddanda et al., 2014).

To demonstrate that excess amounts of orally administered vitamin-E were toxic to human being and several animals such as, rabbits and rats were treated and have showed significantly higher stages of vitamin E (plasma level) than the control (Costagliola et al., 1986). In comparative PK study of the treated and control group, no significant difference has been noticed in the concentration of vitamin E in a various cavities of eye. Lastly, administration of vitamin E administration didn't produce any effect on ascorbic acid concentration present in the fluid of eye cavities.

In rat models, tocotrienol and α-tocopherol derived from palm, helps in the prevention of chronic eye disorders by reducing level of IL-1 and IL-6. Free radicals from agents were responsible for activating the transcription factor NFB, resulting in the production of bone resorbing cytokine (Alwan et al., 2012). Tocotrienol is a more potent antioxidant than α-tocopherol, neutralizing free radicals and prevent activation of NFB, controls the production of cytokine and inflammation.

The effect of corneal entry of riboflavin-5 -phosphate with the help of vit- E TPGS against free-radicals was investigated. Results showed the deeper penetration of riboflavin in the stromal layer that could be the results of supplementation of photo-protection and prevents the free radical production in the photo induced process. *In vitro* procedures showed, TPGS improves corneal entry of corneal penetration, as well stiffness (Ostacolo et al., 2013).

Repaglinide nanocrystal's formulation was developed by Di Cagno and associate. TPGS used for elevating the saturated solubility and it was observed that there were up to 25.7 fold oral bioavailability than the free drug (15.0 fold). Although, it was found that if an unstable drug was loaded into TPGS- micelles, there was an improvement in solubility and stability of formulation (Di Cagno et al., 2012).

TPGS is non-ionic surfactants, besides that, it is commonly utilized in emulsification, solubilisation and nutrition supplement. Mostly drugs that are used in ocular disorder are lipophilic in nature, therefore they are not permeable in the posterior segment

through cornea thus it may enhance the hydrophilic medications like steroids, cyclosporine's, or antibiotics (Constantinides et al., 2006).

TPGS has ability to reduce the internal surface tension between polymer and drug. Hence it has been used as an emulsifying agent that also results in improvement of entrapment efficiency, tissue penetration and good therapeutic effects (*in vivo)* (Fischer et al., 2002).

Vitamin-E TPGS is water soluble in nature. It was prepared by combination of two substances by ester bond between vitamin E and polyethylene glycol. Their use has been associated with the prolonged delivery of drugs of numerous classes (Guo et al., 2013).

Vitamin E loaded contact lenses were formulated and evaluated by Hsu and associates for glaucoma therapy. Vitamin E incorporation was found to delay the release of timolol and dorzolamide for up to 2 days (Hsu et al., 2015).

The impact of vitamin E on the ganglion cell death (RGC) by using a induced glaucoma proto type was performed by Ko and co-workers (Ko et al., 2010). The cell death on ganglion cell was significantly more for groups of rats that feed vit E deficiency diet than the normal diet due to induction of IOP elevation experience.

2.3 Literature review on nanoparticles-based formulation for the treatment of glaucoma

Nepafenac, a nonsteroidal anti-inflammatory (NSAID) drug, which is water-insoluble presented in its suspension form (Nevanac®). Scientists prepared the complex to create an ion-responsive phase transition with sodium alginate and Protanal PH 1033 to increase localisation of drug on the ocular surface and minimizing multiple drug regimens. When the optimized formulation was exposed to simulated tear fluid, its viscosity increased 30-fold (35 °C). *In situ* gel preparation showed enhance permeation, hence more drug travel across cornea then plain eye drop. Furthermore, when compared to Nevanac®, the *in situ* gel preparation had 5-times more concentration than marketed eye drop in aqueous humor. Moreover, in *ex vivo* drug retention was found to be higher in an animal model such as porcine eye perfusion model (Shelley et al., 2018).

Kanamycin (KS) loaded nanoparticles by using PLGA-Vitamin-E-TPGS were prepared. PLGA was added in the formulation for alteration of the surface. The altered surface of NPs was considered to increase adsorption and absorption as well. Finally, it was concluded that modified formulation could be able to produce controlled release formulation; hence it would results in reduction of dosage frequency (Mustafa et al., 2017).

Aburahma and Mahamoud developed novel ocular inserts using BRT. Ocular insert when inserted into albino rabbit eyes, its therapeutic action in terms of intraocular pressure (IOP) lowering effect was excellent than its solution form. Furthermore, the one side coat on ocular insert demonstrated a greater hypotensive effect than its non-coated or coated from both side counterparts. Moreover, the ocular inserts were mucous friendly and sustaining effect (Aburahma and Mahamoud, 2011).

Sah and associate studied the penetration profile of loteprednol etabonate incorporated in PLGA loaded nanoparticles into the excised cornea of goat was studied. The optimised formulation's showed better (F5) entrapment efficiency and mean diameter were determined to be 96.31±1.68 percent and 167.6±0.37 nm, respectively (Sah et al., 2017).

Dorzolamide nanoparticles were formulated by Warsi and associates using different surface active agents such as, PVA and vit-E TPGS (co-surfactant). The combined effect of both the surfactant along with process parameters, on drug encapsulation and size of particles were also studied. Nanoparticles prepared by vit-E TPGS had more entrapment efficiency than PVA nanoparticles and found more effective than conventional eye drop (Warsi et al., 2014).

Optimized novel sinomenine hydrochloride thixotropic system was developed by Song and associates. Optimum formula F2–F3 containing 0.5 percent drug was manufactured and used as gel, while 0.5 drug solution was manufactured and marked as the controlled group. Over 480 min, the gel group provided sustained drug release. There was no toxicity event or irritant effect in any of the studies. The elimination times for the control and gel groups were 10 and 25 min, respectively (Song et al., 2013).

Formulated micro and nanoparticulate based novel ocular drop to improve availability at eye cavity, achieving target tissue, and reduce pulsatile entry of the therapeutic agent on the eye surface was studied (Singh and Shinde, 2010). The nanoparticles would not only extend the drug's residency but would also reduce pulsatile entry in the ocular globe. An animal study revealed that topical instillation of brimonidine tartrate-incorporated nanoparticles to albino rabbits could prolong drug release over an 8 h period, which reduces the number of doses required.

PEG and chitosan coated nanocarriers and their effect on mucoadhesiveness overall ocular mucosa was demonstrated. It was observed that coating had a significant impact on particles physicochemical properties. In the greater sense, as expected, the chitosan coat can increase size of the particles and caused the surface charge to invert (from negative to positive). There was not any alteration of surface charge with PEG-coated and an uncoated nanocapsule. The particles size obtained with PEG-coated nanocapsules was smaller (De Campos et al., 2004).

Nanoparticle based novel eye preparation using chitosan for improvement of ocular availability was prepared. After topical application of Cyclosporin A (CyA) loaded chitosan nanocarrier into the rabbit's eye, therapeutic concentrations in ocular tissues externally were achieved in 2 days, while their negligible concentration of CyA showed in blood, and inner environment of eye. The concentration of novel drug formulations was significantly higher than drug suspension (De Campos et al. 2001).

New ocular drug carriers based on liposome–chitosan nanoparticles (LCS- NP) complexes were developed. Liposomes in suspension ranged in size from 290 to 420 nm and varied according to lipid composition. The findings of this study revealed that nano systems composed of chitosan and phospholipids were stayed in the mucus layer for sufficient duration before entering the conjunctiva of eye (Diebold et al., 2007).

Nanostructured nanoparticles prepared using chitosan was found to improve corneal permeability. These systems have ability to incorporate the drug ranging lipophilic low molecular weight substance for instance, indomethacin. It also facilitates to incorporate the medium molecular weight substances e.g. Cyclosporine A and large molecules (*e.g*. gene.). These nanocarriers can incorporate diverse molecules to

improve ocular penetration, ocular retention and gene transfection (Contreras-Ruiz et al., 2010).

3. Rationale of the Work

BRT, highly selective alpha-2 adrenergic agonist is approved for glaucomatous eyes. Unlike other drugs, it appears to have a dual action i.e. suppression of aqueous flow and an improvement of uveoscleral outflow. Besides that, BRT is a safer, neuroprotective, and preferable prescribed drug in the management of glaucoma. But dose-related adverse effect (hyperaemia/redness) is associated with the BRT. During the treatment, the sign (dryness of the mouth) and symptoms of redness and burning may persist with varying severity for a time and making the patient uncomfortable and worried, which leads to discontinued treatment by the patients. Instillation of eye drop required three times in a day was more effective in reducing intraocular pressure but the rate of compliance decreased as the number of daily doses increased. The generic BRT 0.2% (Bausch & Lomb Pharmaceuticals and Tampa, Florida, USA) prices around $529.34 and $352.89 each year for 2-3 drops each day as eye treatments. Treatment with BRT the daily costs/eye Rs. 3.14±0.03, yearly costs/eye 1147.75±11.15. Our work had the objective of determining the bioavailability of dose proportionality of BRT with and without vitamin E TPGS. Vitamin E has an antioxidant property that potentiates the antiglaucoma action of a drug by improving the outflow of aqueous humor by losing trabecular meshwork cells of glaucomatous eyes. Also, it increases the fluidity of cellular membrane and level of glutathione in the eyes.

Vitamin E TPGS may potentiate the action of the drug by modulating phospho-glycoprotein (P-gp) efflux transport. The dual drug approach might reduce the cost of treatment as well as the adverse effect of the drug. Therefore, the BRT is encapsulated with the vitamin-E TPGS in the carrier system, due to which the carrier easily permeates through the cornea and may increase bioavailability. To improve drug residency time on eye and improve the viscosity of nanodispersion, nanoparticulate carrier system was incorporated into *in situ* gel system. Consequently, glaucoma patients are more likely to adhere to the dual approach due to minimal dose frequency and dose-related effects.

3.1 Rationale for selection of formulation approaches

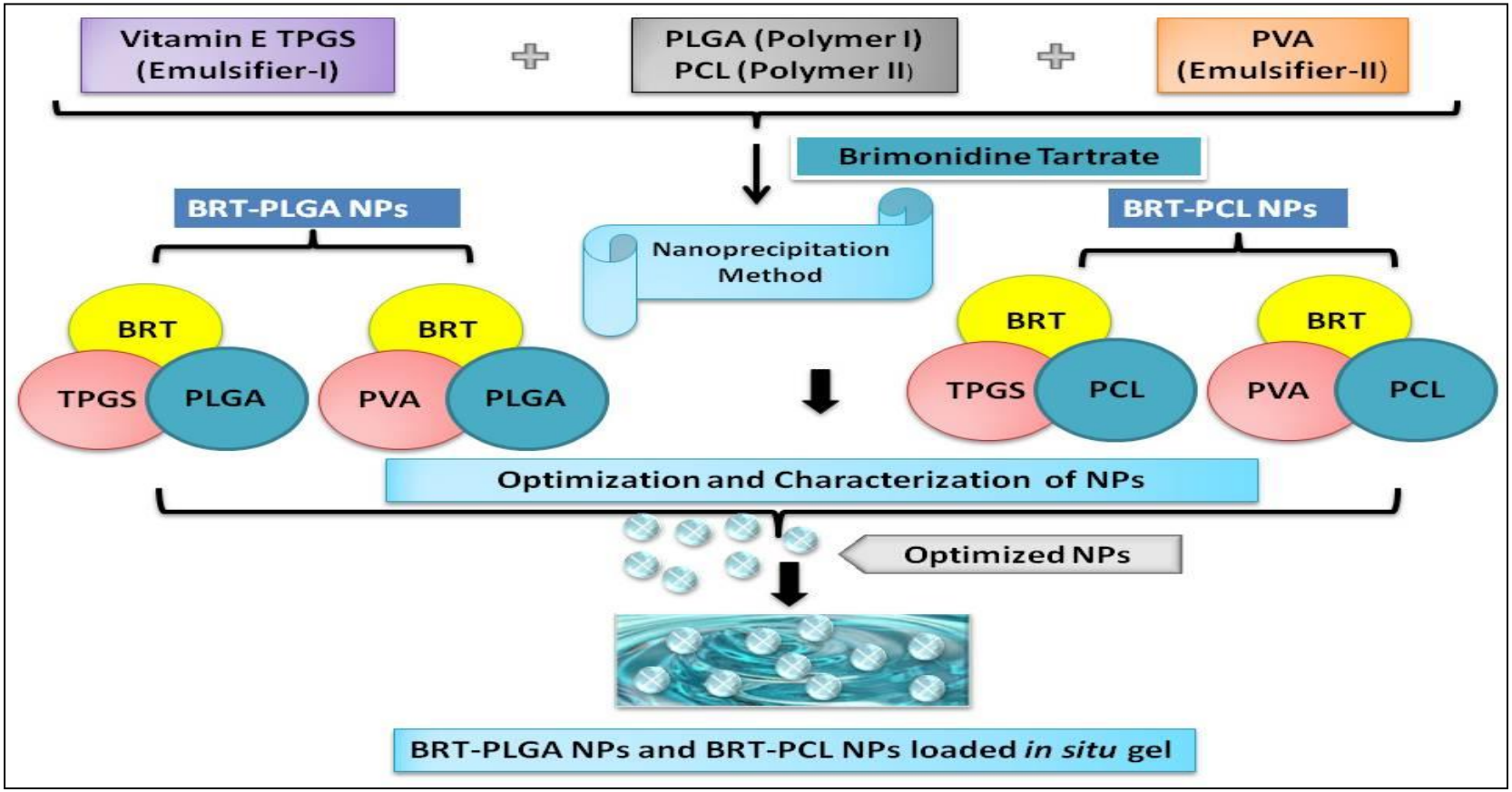
Following are the rationale for selection of formulation approaches

- To improve the permeability of eye drop through the tight junctions of cornea.
- To increases the precorneal retention time with poor patient compliance.
- To evaluate the bioavailability of the dose proportionality of BRT with and without vitamin E TPGS.
- To reduces the adverse effect of BRT by reducing the dose of drug and also co-administration with vitamin E TPGS.

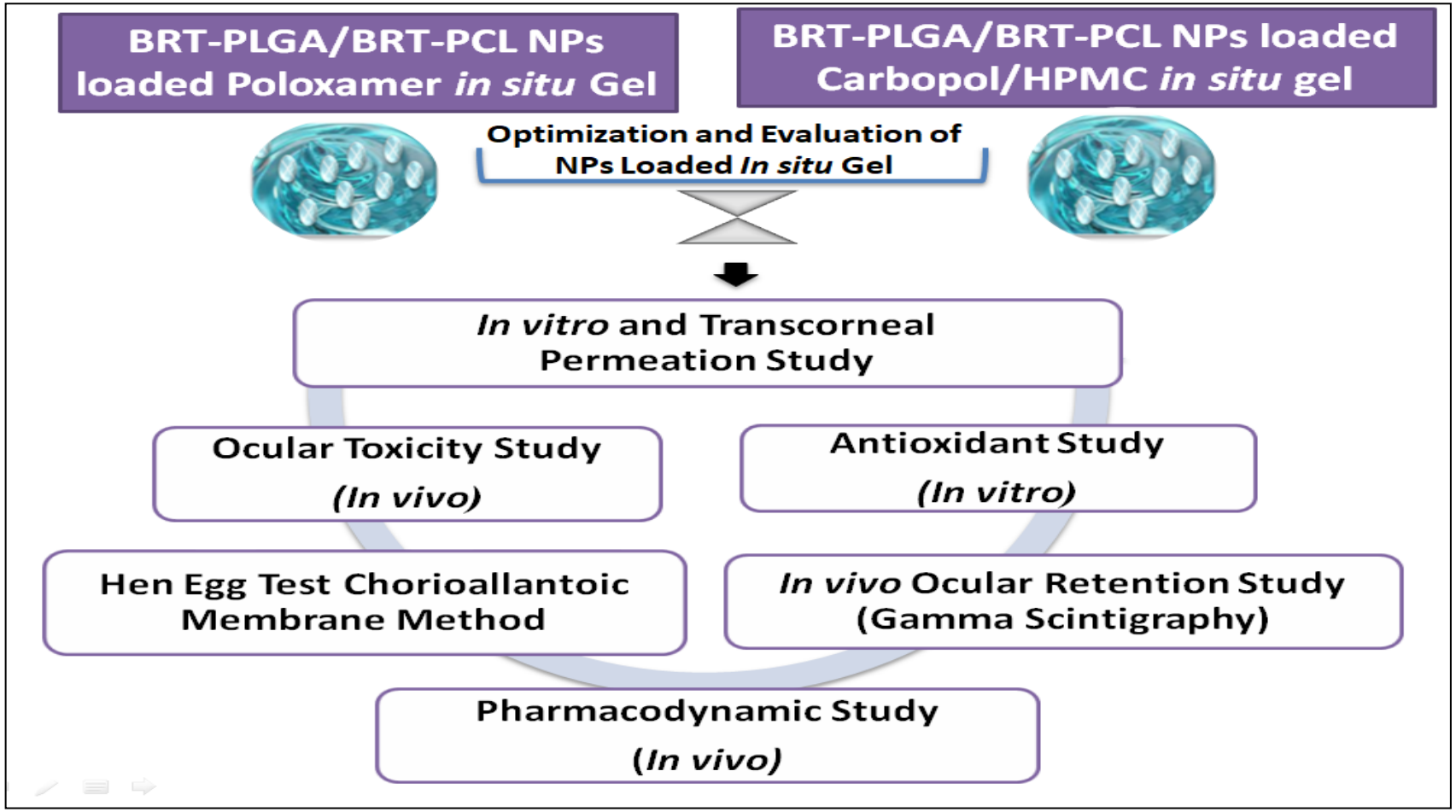
WORK SCHEME

Following work scheme have been used for the formulation preparation

Scheme-I



Scheme-II


BRT-PLGA/BRT-PCL NPs loaded Poloxamer *in situ* Gel
BRT-PLGA/BRT-PCL NPs loaded Carbopol/HPMC *in situ* gel
Optimization and Evaluation of NPs Loaded *In situ* Gel
In vitro and Transcorneal Permeation Study
Ocular Toxicity Study *(In vivo)*
Antioxidant Study *(In vitro)*
Hen Egg Test Chorioallantoic Membrane Method
In vivo Ocular Retention Study (Gamma Scintigraphy)
Pharmacodynamic Study *(In vivo)*

4. Aim and Objectives

For the purpose of treatment, the different regions in eyes, new drug carrier's system in the form of nanoparticles will be prepared using an antiglaucoma agent with vitamin E derivatives and platform technology will be developed to manufacture well-defined, stable nanoparticles via a reproducible and versatile technique, which would allow the accommodation of a variety of therapeutic agents for the glaucoma management.

Ocular preparations mostly eye drops are limited to permeate and absorbed due to the ocular barrier (Gratieri et al., 2010). These barriers may be present in different part of the eye like tear film, cornea, conjunctiva and retina which may restrict the entry of the drug molecule from anterior to the posterior site (Gajra et al., 2012).

The smaller size of molecule and lipophilic nature of drugs are the main two major factors that affect the permeability of the drug through cornea. This offers another advantage of dose reduction and decreased adverse effect profile (Makadia and Siegel, 2011).

In addition, polymers (PLGA/PCL) based nanoparticles are mucoadhesive in nature, which help in binding of the drug on the cornea and also does not drainage the drug through tear (Warsi et al., 2014). Moreover, above ophthalmic based drug delivery methods have demonstrated relatively long stability and rapid clearance.

To address these issues, other methods for increasing medication efficacy are to increase the residence duration of ocular preparations utilizing thermosensitive *in situ* gel (Morsi et al., 2016). Due to a change in a unique physicochemical attribute on ocular surface such systems showed better stability and sol-to-gel phase changes. After being instilled into the cul-de-sac at 37 ºC, this system undergoes rapid gelation due to the presence of thermosensitive polymer (Poloxamer) and provides sustained drug release.

In present study BRT PLGA-TPGS and PCL-TPGS nanoparticles were introduced into thermosensitive *in situ* gel systems in this study, resulting in prolonged release and improved drug permeability, and making a significant contribution in the clinical phase.

The objectives of present study are:

- To develop and evaluate a smart PLGA-Vit E TPGS and PCL-Vit E TPGS nanoparticles of BRT for the treatment of glaucoma.

- Inclusion of polymer-based vit E TPGS nanoparticles loaded into a thermosensitive *in situ* gel system.
- Pharmacokinetic and Pharmacodynamic studies of optimized formulation.

5. Plan of Work

a) Characterization of drug: The drug will be characterized for its authenticity on the basis of physical properties, spectral analysis (FTIR spectroscopy, UV spectrophotometry etc.)

b) Analytical method development:. UV spectrophotometry method will be used for the analysis for the drug.

c) Preformulation studies: Under the Preformulation studies, several aspects related with drug, excipients and formulation development will be studied such as:

- **Determination of solubility of drug in various solvents:** Solubility is a characteristic physical property referring the solute dissolve in a solvent. It is measured in terms of the maximum amount of solute dissolved in a solvent at equilibrium.
- **Partition Coefficient of the drug:** Partition Coefficient will be determined by using n-octanol/isotonic phosphate buffer of pH 7.2 using shake flask method.
- **Drug excipient interaction study:** Physical mixture of the drug and excipients will be prepared at different temperature and humidity conditions and the assay of drug will be carried in order to ascertained any chemical interaction.

d) Preparation and optimization of brimonidine nanoparticles: The preparation of nanoparticles by Nanoprecipitation method. The optimization will be done by using box-behnken expert design.

e) Characterization of drug loaded nanoparticles. Nanoparticles will be characterized for

- Particle Size
- Zeta potential
- Polydispersibility index
- Entrapment Efficiency
- Surface morphology by TEM

f) **Preparation, optimization and evaluation of brimonidine nanoparticles loaded *in-situ* gel:** The preparation of nanoparticles loaded *in situ* gel will be done by cold method. Optimization and evaluation of *in-situ* gel based on pH, gelling time, gelling temperature *etc.* will be performed.

g) ***In-vitro* release studies:** It will be determined by using dialysis tube technique and franz diffusion.

h) ***In-vitro* trans-corneal permeation studies:** *In vitro* trans-corneal permeation studiesusually done on the goat cornea, collected from the slaughtered house, cornea will be removed and mounted on Franz diffusion cell.

i) **Ocular Irritation study:** Rabbits will be then observed for conjuctival discharge, chemosis and redness. Ocular irritation index will be calculated as an indicator of clinically significant irritation**.**

j) **Pharmacodynamics and Pharmacokinetic studies**. These studies will be done on rabbit's eye as animal model. The intraocular pressure will be measured of concentration different using tonometer at different time interval. The different groups of the rabbit's will be made and optimized formulations will be tested and compared the amount of drug concentration in aqueous humor with the placebo and marked conventional delivery systems of the drug.

6. Expected outcomes

Brimonidine tartrate (BRT) is a hydrophilic anti-glaucoma medicine available in solution form (Brimopress Eye Drops®) in the market. The improved PCL/PLGA and vitamin E TPGS based formulations were studied and evaluated. BRT-PLGA NPs were formulated, and it was observed that the size of NPs was in the range. Surface morphology studies and DSC revealed that BRT-PLGA NPs exhibited a smooth and spherical morphology. From these findings, it can be said that the thermo- responsiveness of poloxamer gel is considerably relied on concentration. Higher amount of poloxamer 407 leads to rapid gelation, but at lower gelation temperature. Results showed that gels exhibit greater network and showed rigidity at fixed Poloxamer concentration. In case of BRT-PLGA-NPs loaded carbopol/HPMC based in situ gelling system all formulations were clear and colorless but little translucent due to the opalescent nature of nanodispersion. The highest cumulative release rate of the drug in simulated aqueous humour over 24 h was confirmed in an in vitro evaluation. The developed in situ gel showed a

higher ix permeation rate through the goat eye 51.18 µg.cm-2 .h-1 (PCL NPs) and 50.24 µg.cm-2 .h-1 (PLGA NPs) in 4 h whereas, marketed eye drop showed 7.618 µg.cm-2 .h-1 and BRT in situ gel showed 20.56 µg.cm-2 .h-1 transcorneal flux in 4 h. In addition, novel optimized in situ gel showed sustained action and continuously reduction of IOP till 12 h. The optimized preparation also studied for in vivo ocular irritability and tolerability indicates no signs of discomfort. In vivo ocular irritancy test was performed in the rabbit eye to check the dose related hyperaemia of BRT. Slight redness has been observed in rabbit eyes with not only the marketed eye drop but also finally optimized formulation of BRT (0.1% w/v). Therefore, the reduced concentration of BRT i.e.0.05% and 0.025% was prepared using previously optimized formula. Percent reduction of IOP of 0.05% and 0.025% of BRT NPs based in situ gel showed 34.37±4.21% and 13.61±3.01% respectively till 4 h. In conclusion, all the objectives of the study were met by successfully developing novel formulation of BRT for management of glaucoma.

7. References

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